oxysterol as a potential target for antiviral therapy. This idea is particularly intriguing as 25HC administration restored immune cell numbers in an HIV infection model. Additionally, the inhibitory effect of statins against viruses such as HCV (Ye et al., 2003) demonstrates that modulation of sterol and isoprenoid biosynthesis pathways stands as a potential mechanism for antiviral therapy using existing therapeutic drugs.

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Inflammation Makes T Cells Sensitive

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Inflammatory cytokines shape CD8$^+$ T cell responses. In this issue of *Immunity*, Richer et al. (2013) and Raue et al. (2013) demonstrate that inflammatory cytokines dynamically fine-tune antigen sensitivity of CD8$^+$ T cells to potently detect and better eliminate infected cells.

Productive CD8$^+$ T cell responses are critical to immune defense against invading pathogens. In order to activate and tune CD8$^+$ T cell responses, cognate triggering of the T cell receptor (TCR) and concurrent ligation of CD28 is not sufficient, an additional third signal in the form of inflammatory cytokines is required for the differentiation of fully functional effector CD8$^+$ T cells (Curtsinger and Mescher, 2010). Naive CD8$^+$ T cells exit the thymus with the potential to differentiate into a spectrum of effector populations possessing unique capacities for survival, proliferation, cytokine expression, cytotoxicity, and memory formation. It is well appreciated that these defining characteristics of the T cell response are heavily influenced by local cytokines at the time of priming. Currently, there is great interest in understanding how distinct inflammatory cytokines direct and tune CD8$^+$ T cell responses and the dynamic impact they have throughout the course of infection.

It has been previously recognized that as an infection progresses, pathogen-specific CD8$^+$ T cells exhibit enhanced sensitivity to antigen (Slifka and Whitton, 2001). Considering that T cells do not mutate their T cell receptor, how the enhanced sensitivity is achieved has remained unclear. In this issue of *Immunity*, Richer et al. (2013) and Raue et al. (2013) address this question. Richer et al. (2013) demonstrate the important role the inflammatory milieu plays in tuning antigen sensitivity of CD8$^+$ T cells via the enhancement of proximal TCR signal transduction. This finding suggests an elegant mechanism by which the sensitivity of local T cells is enhanced at sites of pathogenic assault with limited potential for systemic activation and potential distant autoimmunity. Raue et al. (2013) reveal the capacity of two inflammatory cytokines, interleukin-12 (IL-12) and IL-18, to initiate an effector program in memory CD8$^+$ T cells in the absence of antigen, thereby giving CD8$^+$ T cells a head start on recurring pathogens and potentially accounting for some of the rapid responsiveness to reinfection. Both of these articles suggest a mechanism whereby the immune response at the site of invasion is given a selective advantage and provide insight toward our understanding of how CD8$^+$ T cell function is regulated to identify and kill infected cells at the beginning of infection when antigen is limited (Figure 1).

Although the profound capacity of inflammatory cytokines to drive proliferation and effector differentiation during the priming of naive CD8$^+$ T cells has been well defined (Curtsinger and Mescher, 2010), the impact of these cytokines on fine-tuning antigen sensitivity

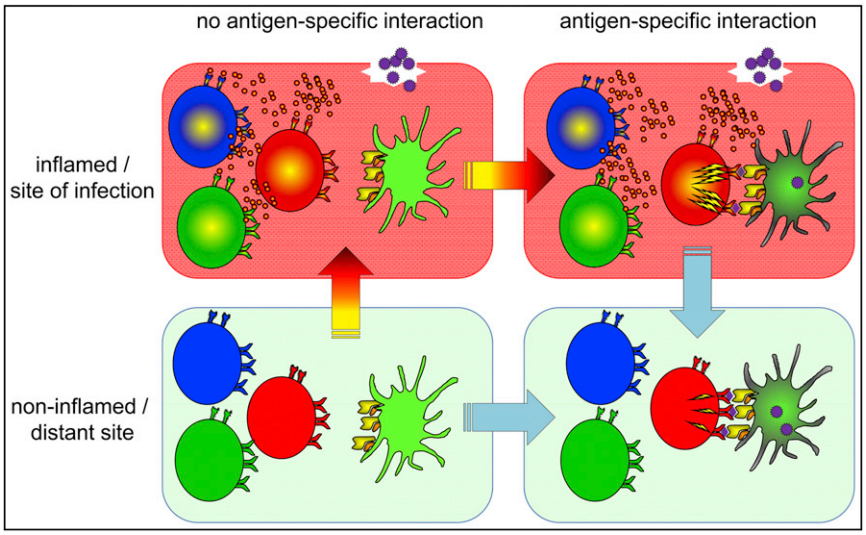

Figure 1. Dynamic Remodeling of CD8 T Cell Sensitivity to Antigen at the Site of Infection by Inflammatory Cytokines

Bottom panels show that in the absence of inflammatory cytokines, such as tissues distant from the site of virus infection, circulating naïve and memory T cells are not sensitized to the virus (lower panels). Even when antigen-specific interactions occur (red T cell), TCR signaling is decreased and higher amounts of antigen are required to stimulate the T cells (bottom right panel). Top panels show that at the site of virus infection, inflammatory cytokines are produced (including type I interferons, IL-12 and IL-18), sensitizing all naïve and memory CD8 T cells regardless of antigen specificity, leading to enhanced effector potential (i.e., cytotoxic activity and cytokine production) in the area. Inflammatory cytokines also enhance the antigen sensitivity of naïve and memory T cells, enabling them to detect the low levels of infection present early in infection and transduce much stronger TCR signals to the lower amount of antigen (top right panel). Importantly, although all the CD8 T cells are sensitized, ligation of the TCR is required to enact the enhanced effector functions instilled by exposure to inflammatory cytokines. Upon leaving the site or resolution of infection, the same sensitized T cells return to their baseline state (bottom left panel).

and whether this attribute is then permanently hard-wired into the cells has not been explored. In the current study, Richer et al. (2013) modulate antigen concentration by pulsing dendritic cells with titrated amounts of ovalbumin (DC-OVA). DC-OVA are then used to stimulate $CD8^+$ T cell responses in either uninfected control mice, *Listeria monocytogenes* (LM) infected, or lymphocytic choriomeningitis virus (LCMV)-infected animals—systems chosen because of the multiple and somewhat different cytokine inflammatory responses induced by these pathogens. By using the same stimulating DC and noninfection related antigen, the authors can separate antigenic stimulation from effects of the inflammatory milieu (although it should be noted that the stimulating DC themselves may have different life spans, home to different lymphoid and effector tissues, or distinct areas within tissue, or be functionally altered based on residing in the different immune environments). Robust cytokine responses that include IL-12 and type I interferons (IFN-I) are stimulated by LM, whereas the response to LCMV is predominantly IFN-I mediated. Five days after DC-OVA immunization, OVA-specific $CD8^+$ T cell responses in LM-infected mice were markedly more sensitive to low concentrations of OVA peptide compared to OVA-specific $CD8^+$ T cells stimulated in uninfected animals. This effect was even more pronounced in LCMV infection. Using OVA-specific $CD8^+$ T cells unable to respond to IL-12 or IFN-I signaling, Richer et al. (2013) show that both IL-12 and IFN-I enhance $CD8^+$ T cell intrinsic antigen sensitivity. This enhancement was evident in polyclonal endogenous $CD8^+$ T cell responses to OVA as well as in monoclonal populations of TCR transgenic OT-I cells, the latter demonstrating the intrinsic tuning of the cells separate from selection of higher affinity T cell clones. In addition to the heightened sensitivity to antigen density, OVA-specific T cell responses initiated in the presence of LM and LCMV produced higher amounts of IFN-γ at the single cell level and possessed enhanced cytolytic activity.

These findings are consistent with results described by Raue et al. (2013) where memory LCMV-specific $CD8^+$ T cells cultured for 5 hr with a combination of IL-12 and IL-18 demonstrated enhanced antigen-independent proliferation, cytokine production, and cytotoxicity in vitro. Further, adoptive transfer of IL-12 + IL-18-treated memory $CD8^+$ T cells decreased LCMV titers 65%–85% compared to mice receiving memory $CD8^+$ T cells treated with media alone, supporting the assertion that inflammatory cytokines substantially enhance the function of responding $CD8^+$ T cells at the site of infection and providing a mechanism by which rapid recall responses are enacted.

Infection-associated inflammation is closely linked both spatially and temporally to pathogen replication, as the amounts of inflammatory cytokines wane as the infection is resolved, preventing excessive immunopathology or autoimmunity. Accordingly, Richer et al. (2013) demonstrate that the enhanced antigen sensitivity induced by the inflammatory environment in both naive and memory $CD8^+$ T cells diminishes as the infection is resolved. When DC-OVA were administered 8 days after infection, enhanced antigen sensitivity and IFN-γ production was still observed, albeit at a much-reduced magnitude. Further, increased antigen sensitivity of memory $CD8^+$ T cells was observed 4 days after LCMV infection; however, by day 14 after infection the increase in sensitivity was no longer evident. Consistent with the need for active inflammatory cytokine signaling to sustain heightened antigen sensitivity, Raue et al. (2013) observed that IL-12+IL-18 pretreated memory $CD8^+$ T cells ceased proliferation upon transfer into uninfected mice. Together these findings establish the important point that the responses are not hard-wired (as are other parameters of T cell programming [Kaech and Ahmed, 2001]) and that antigen sensitivity is actively tuned by the ongoing infection. Once purged, the pathogen-specific cells return to a less-sensitive state, thereby attenuating hyper-aggressive cells and limiting potential autoimmunity at the site of infection. Thus, the enhancement of immunity in the context of active infection

is a tightly controlled, highly tunable event, dependent on close proximity to infection.

There is an active interest to understand the specific molecular mechanisms through which inflammatory cytokines enact their effects on responding CD8+ T cells. A recent comparative report of gene expression profiles between $CD8^+$ T cells activated in the presence of IL-12 or IFN-I revealed sets of genes that were regulated analogously and uniquely by each cytokine and that could therefore differentially guide ensuing immune responses in a pathogenic-specific manner (Agarwal et al., 2009). In the current study, Richer et al. (2013) provide a functional impact of inflammatory cytokines in promoting pathogen-specific immunity. The authors observe that LCMV-induced inflammatory cytokines do not specifically increase expression of important signaling components downstream of the TCR but instead boost their activity. The concurrent presence of LCMV-induced inflammatory cytokines significantly enhanced the phosphorylation of Zap-70 and PLCγ by OVA-specific $CD8^+$ T cells following TCR stimulation resulting in heightened activation of ERK proteins. Importantly, this effect was dependent upon TCR engagement because ERK phosphorylation was not increased in OVA-specific $CD8^+$ T cells from LCMV-infected mice stimulated with PMA+ionomycin. Consistently, IFN-γ amounts were substantially higher when cells were restimulated with OVA peptide as compared to PMA+ionomycin. These results highlight a mechanism to safeguard against inappropriate activation, wherein exposure to inflammatory cytokines tunes antigen sensitivity of all $CD8^+$ T cells in a local area (naive and memory) but requires cognate interactions to enact the enhanced effector functions.

Together, these studies suggest a mechanism whereby naive and memory T cells at the site of infection will have a selective advantage without arousing the immune response systemically. Thus by locally tuning responses the immune system is able to induce a potentially dangerous readiness to ensure sensitive identification and rapid killing of the pathogen without the risk of nonspecific or excessive immunopathology where the pathogen is not. Further, the fine-tuning of antigen sensitivity of localized responses may further account for the faster activation and clearance of reinfection by memory $CD8^+$ T cells, distinct from their enhanced precursor frequency and separate from programmed changes in the cells themselves.

The observations of Richer et al. (2013) and Raue et al. (2013) contribute to our growing understanding of how $CD8^+$ T cell responses are modulated in the context of concurrent infection and are highly relevant to clinical applications of adoptive T cell and cytokine adjuvant therapies. These studies clearly demonstrate that the antigen sensitivity threshold by $CD8^+$ T cells is markedly lowered prior to antigen recognition in the presence of inflammatory cytokines. However, the mechanisms and interplay (both cellular and molecular) linking inflammatory cytokines to remodeling of T cell function at the initiation of infection and how individual cytokines distinctly tune T cell activity to respond optimally to a precise pathogen remain to be elucidated. Further, naive and memory $CD8^+$ T cells are clearly different entities, so how these cytokines shape antigen sensitivity to impact their long-term development is critical for the design of vaccination strategies to elicit optimal immunity against a specific pathogen. Ultimately, understanding the balance between effector and memory T cell responses and the signals involved in optimizing both at the site of infection are critical areas of future research.

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